and ileum of the guinea pig. Support of this work by the Verband der Chemischen Industrie, Fonds der Chemischen Industrie, is greatly acknowledged.

Registry No. 1, 73278-98-5; 2, 73279-05-7; 5, 98078-91-2; 6, 117078-41-8; 7, 140872-98-6; 8, 140873-00-3; 9, 140873-02-5; 10, 140873-03-6; 11, 140873-04-7; 12, 140873-06-9; 13, 140873-07-0; 14, 140900-94-3; 15, 140873-09-2; 16, 140873-10-5; 17, 140873-12-7; 18, 140873-13-8; 19, 140873-14-9; 20, 1493-27-2; 21, 28166-06-5; 22a, 140873-15-0; 22b, 140873-16-1; 23a, 140873-17-2; 23b, 140873-18-3; 24a, 140873-19-4; 24b, 140873-20-7; 25a, 140873-21-8; 25b, 140873-22-9; 26a, 140873-23-0; 27e, 150-13-0; 27f, 2122-63-6; 27g, 2122-61-4; 31c, 140873-24-1; 31d, 140873-25-2; 31e, 140873-27-4; 31f, 126632-01-7; 31g, 140873-28-5; 31h, 126632-02-8; 31i, 140873-29-6; 31k, 140873-30-9; 31l, 140873-31-0; 31m, 140925-90-2; 32c, 140873-32-1; 32d, 140873-33-2; 33a, 140873-34-3; 33c, 140873-35-4; 33d, 140873-36-5; 33e, 140873-37-6; 33f, 140873-38-7; 33g, 140873-39-8; 33i, 140873-40-1; 34c, 140873-41-2; 34d, 140873-42-3; 35c, 140873-44-5; 35e, 140873-46-7; 36c, 140873-48-9; 37c, 140873-50-3; 38c, 140873-51-4; 39c, 140873-52-5; 40c, 140873-53-6; 41c, 140900-95-4; 42m, 140873-54-7; 43c, 140873-55-8; 44c, 140873-57-0; 45c, 140873-59-2; 46c, 140873-60-5; 46e, 140873-61-6; 47, 98-09-9; 48, 98-59-9; 49c, 140873-62-7; 49n, 140873-63-8; 50c, 140873-64-9; 50n, 140873-65-0; 51, 94-09-7; 52, 62875-84-7; 53, 5400-81-7; $PhCH_2NMe_3^+[ICl_2]^-$, 114971-52-7; $H_2N(CH_2)_2NH_2$, 107-15-3.

Synthesis, Configuration, and Calcium Modulatory Properties of Enantiomerically Pure 5-Oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylates[1]

Ulrich Rose*,† and Martin Dräger‡

Institut für Pharmazie der Johannes Gutenberg-Universität Mainz, Federal Republic of Germany, and Institut für Anorganische und Analytische Chemie der Johannes Gutenberg-Universität Mainz, Saarstr. 21, D-6500 Mainz, Federal Republic of Germany. *Received February 25, 1991*

Enantiomerically pure hexahydroquinolinones of the structural type **9** were prepared by a variation of the Hantzsch synthesis in which an optically active acetoacetate served as a chiral auxiliary reagent. Determinations of the de and ee values are described. The absolute configurations of the optically pure products were characterized by single-crystal X-ray analysis. The antipodes **9a** and **9b** exhibited calcium antagonistic activities on smooth musculature; the (*S*)-(−)-enantiomer **9b** was the more potent compound with regard to the EC_{50} values which differed by a factor of 100; the intrinsic activity of **9b** was 1.2, compared with a value of 0.54 for **9a**. On the other hand, *R*-(+)-**9a** exerted positive inotropic effects on electrically stimulated atria. The cause of these effects is discussed.

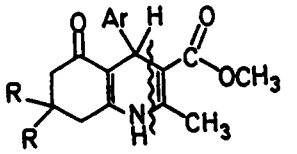
In contrast to the effects of known calcium channel blockers of the nifedipine-type, the so-called calcium agonists, such as Bay K 8644 and CGP 28392, increase calcium influx by binding at the same receptor regions.[2-5] As a physiological consequence, positive inotropic, chronotropic, and vasoconstrictive activities have been observed in vitro and in vivo.[6,7]

An essential prerequisite for a therapeutic application in cases of cardiac insufficiency, however, is cardioselectivity. Investigations on structure–activity relationships with regard to "agonism–antagonism" still do not allow any unequivocal conclusions to be drawn about the molecular requirements for selectivity.[8]

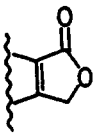
Our previous work in this area was to fix the carbonyl groups in an antiperiplanar position by anellation at the dihydropyridine ring, since it had been proposed[9] that the quality of action is associated with the respective position of the ester carbonyl group.

The racemic hexahydroquinolinones **10** and **11**[10-12] as well as the 5-oxoindeno-1,4-dihydropyridines **12**[13] exhibited simultaneous calcium antagonistic effects on smooth musculature and positive inotropic activities on electrically stimulated left atria of guinea pigs. Furthermore, the anellated lactone **11** effected an increase in contractility of isolated ventricular papillary muscle.[14]

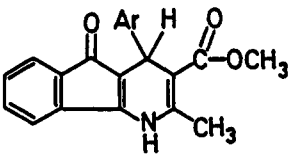
It is now known that the dihydropyridine (DHP) receptors exhibit stereospecificity: the optical antipodes of asymmetrical dihydropyridines often possess not only differing receptor affinities[15] but sometimes also generate opposing effects.[16,17] To better differentiate the above-described anellated structures, further synthetic work was carried out to prepare the optical antipodes of hexahydroquinolinones.



10



11



12

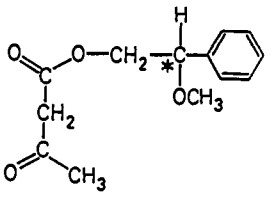
† Institut für Pharmazie.
‡ Institut für Anorganische und Analytische Chemie.

(1) Presented in the 11th International Symposium on Medicinal Chemistry, Jerusalem (Israel), September 1990.
(2) Hess, P.; Lansman, J. B.; Tsien, R. W. Different Modes of Ca Channel Gating Behaviour Favoured by Dihydropyridine Ca Agonists and Antagonists. *Nature* 1984, *311*, 538–544.
(3) Schramm, M.; Thomas, G.; Towart, R.; Franckowiak, G. Activation of Calcium Channels by Novel 1,4-Dihydropyridines: A New Mechanism for Positive Inotropics on Smooth Muscle Stimulants. *Arzneim.-Forsch.* 1983, *33*, 1268–1272.
(4) Schramm, M.; Thomas, G.; Towart, R.; Franckowiak, G. Novel Dihydropyridines with Positive Inotropic Action through Activation of Ca^{2+} Channels. *Nature* 1983, *303*, 535–537.
(5) Loutzenheiser, R.; Rüegg, U. T.; Hof, A.; Hof, R. P. Studies on the Mechanism of Action of the Vasoconstrictive Dihydropyridine, CGP 28392. *Eur. J. Pharmacol.* 1984, *105*, 229–237.
(6) Towart, R.; Schramm, M. Recent Advances in the Pharmacology of the Calcium Channel. *Trends Pharmacol. Sci.* 1984, *5*, 111–113.

0022-2623/92/1835-2238$03.00/0

Chemistry

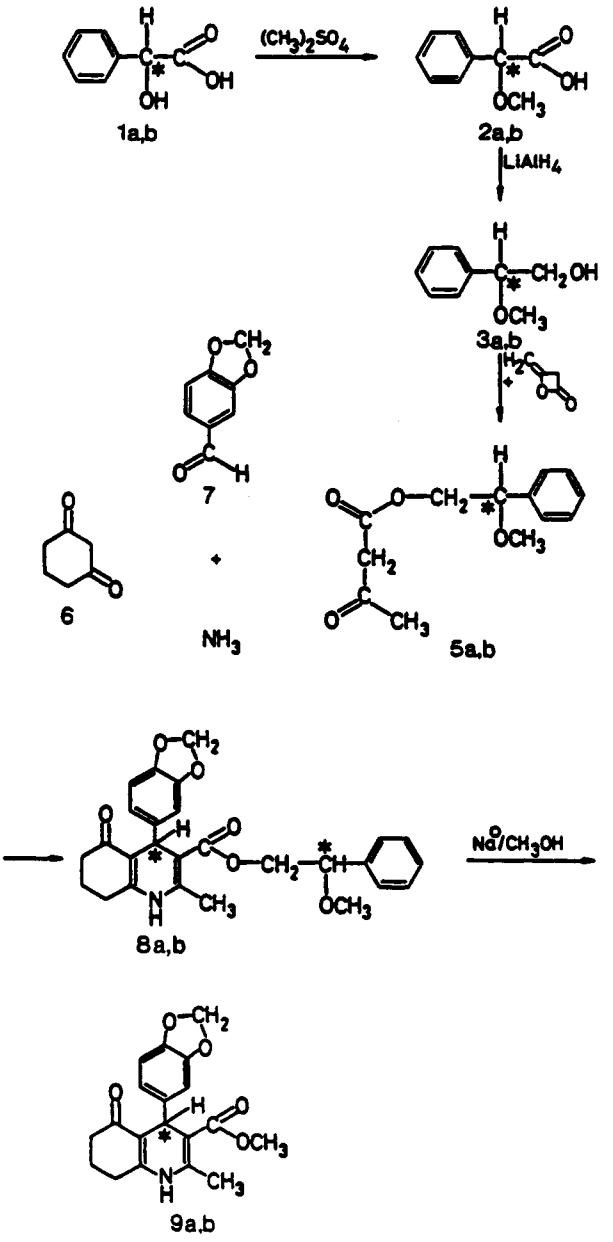
Since a classical isomer separation by way of diastereomeric salts was not possible because of the absence of sufficiently acidic or basic centers in these structures, the desired objective had to be realized by an asymmetric synthesis. In order to achieve an optical induction at the asymmetric center to be formed (C-4), an acetoacetate ester with an optically active alcohol side chain was chosen as the chiral auxiliary reagent (5).[18]



5

A retrosynthetic analysis indicated that the inductor 5 should be accessible from the reaction of diketene (4) with the respective chiral alcohol 3. Hence, mandelic acid, either as its racemate or preferably, as the (*R*)- or the (*S*)-isomer, should be a suitable starting material for construction of the corresponding alcoholic side chain. The use of racemic mandelic acid would make it necessary to carry out an antipode separation at the stage of the starting material or of an intermediate. In any case, methylation of mandelic acid is an indispensable precondition since, otherwise, an unambiguous reaction of the reduction product with diketene would not be possible as a result of the presence of two alcoholic groups. First of all, the

Scheme I



synthesis of that enantiomer or diastereomer which can be obtained starting from (*R*)-(−)-mandelic acid (1a) will be described. According to a method reported by Braun[19] or the modification reported by Reeve,[20] (*R*)-(−)-mandelic acid was methylated with dimethyl sulfate in aqueous sodium hydroxide solution under thermal control of the reaction. After completion of this exothermic reaction, the isolated and purified methoxyphenylacetic acid (2a) exhibited an optical rotation of $[\alpha]^{20}_{D} = -117°$ (literature values: −140° and −144°);[21,22] the optical purity of the product was thus only 83.6 or 81.3%, respectively. The observed racemization can be explained by the fact that the exothermic reaction takes place practically at the asymmetric center and, accordingly, the degree of racemization increases with increasing temperature. In order to avoid this first sensitive step, racemic mandelic acid was methylated by the method described above, and a subse-

(7) Laurent, S.; Kim, D.; Smith, T. W.; Marsh, J. D. Inotropic Effect, Binding Properties, and Calcium Flux Effects of the Calcium Channel Agonist CGP 28392 in Intact Cultural Embryonic Chick Ventricular Cells. *Circ. Res.* **1985**, *56*, 676–681.

(8) Triggle, D. J.; Langs, D. A.; Janis, R. A. Ca^{2+} Channel Ligands: Structure-Function Relationships of the 1,4-Dihydropyridines. *Med. Res. Rev.* **1989**, *9*, 123–180.

(9) Fossheim, R. Crystal Structure of the Dihydropyridine Ca^{2+} Antagonist Felodipine. Dihydropyridine Binding Prerequisites Assessed from Crystallographic Data. *J. Med. Chem.* **1986**, *29*, 305–307.

(10) Rose, U. Kalzium-Modulatoren vom Typ Anellierter Dihydropyridine, Synthese und Pharmakologische Wirkung. *Arzneim.-Forsch.* **1989**, *39*, 1393–1398.

(11) Rose, U. Hexahydrochinolinone mit Calciummodulatorischem Effekt-Synthese und Pharmakologische Wirkung. *Arch. Pharm. (Weinheim, Ger.)* **1990**, *323*, 281–286.

(12) Rose, U. Vinylogous and Coupled Hexahydroquinolinones and Dihydropyridines with Calcium Modulating Effects. *Pharm. Acta Helv.* **1990**, *65*, 178–185.

(13) Rose, U. 5-Oxo-1,4-dihydroindenopyridines: Calcium Modulators with Partial Calcium Agonistic Activity. *J. Heterocycl. Chem.* **1990**, *27*, 237–242.

(14) Rose, U. Synthesis and Pharmacological Activity of Calcium Modulatory Hexahydroquinolinones. *Arzneim.-Forsch.* **1990**, *41*, 199–203.

(15) Bellemann, P.; Schade, A.; Towart, R. Dihydropyridine Receptor in Rat Brain Labeled with ^{3}H-Nimodipine. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 2356–2360.

(16) Hof, R. P.; Rüegg, R. T.; Hof, A.; Vogel, A. Stereoselectivity at the Calcium Channel. *J. Cardiovasc. Pharmacol.* **1985**, *7*, 689–693.

(17) Franckowiak, G.; Bechem, M.; Schramm, M.; Thomas, G. The Optical Isomers of the 1,4-Dihydropyridine Bay K 8644 Show Opposite Effects on Ca Channels. *Eur. J. Pharmacol.* **1985**, *114*, 223–226.

(18) Bayer AG (Wehinger, E., Meier, H., Bossert, F., Vater, W., Towart, R., Stoepel, K., Kazda, S.), Ger. Offen. 2935451, March 19, 1981; *Chem. Abstr.* **1981**, *95*, 42922n.

(19) Von Braun, J.; Anton, E.; Weissbach, K. Die Methylierung des Alkoholischen Hydroxids vom Standpunkt der Elektronentheorie. *Ber. Dtsch. Chem. Ges.* **1930**, *63*, 2847.

(20) Reeve, W.; Christoffel, J. Reaction of Styrene Oxide with Methanol. *J. Am. Chem. Soc.* **1950**, *72*, 1480–1483.

quent enantiomeric separation by way of the diastereomeric ephedrine salts according to Neilson[21] was performed. The levorotatory acid was then liberated in optically pure form ($[\alpha]^{20}_D = -142.5°$) from the well crystallizing salt of the ephedrine base with (*R*)-(−)-methoxyphenylacetic acid (Scheme I).

Compound **2a** was reduced with lithium aluminum hydride to the corresponding alcohol **3a**.[22] After flash chromatographic purification, this configurationally homogeneous product was reacted in the next step with an equimolar amount of diketene or the stable diketene–acetone adduct under sodium acetate catalysis to produce the desired acetoacetate **5a** (Scheme I). This acetoacetate, which contained an asymmetrical center in the side chain, served to effect optical induction at C-4 in a Hantzsch reaction[8-10] for hexahydroquinolinone synthesis (Scheme I).

The diastereomer **8a** was obtained with a high asymmetric induction (de >98%, $[\alpha]^{20}_D = +59.5°$) by reaction of **5a** with cyclohexanedione (**6**), 3,4-(methylenedioxy)-benzaldehyde (**7**), and ammonia in ethanolic solution. Solely the high diastereomeric purity at this stage made possible the preparation of the enantiomerically pure final products by way of cleavage of the optical inductor through transesterification in methanol with metallic sodium (Scheme I). The final product **9a**, as the dextrorotatory enantiomer with an optical rotation of $[\alpha]^{20}_D = +148.6°$ (DMSO, $c = 1.0$), was obtained from this reaction with a yield of 67.4%.

Since the (*S*)-(+)-antipode of methoxyphenylacetic acid cannot be obtained with sufficient optical purity by the above described ephedrine salt crystallization, the first mentioned method was used for the synthesis of (+)-**2b**. Racemization during the methylation of (*S*)-(+)-mandelic acid (**1b**) was finally avoided to a great extent by extremely careful control of the reaction temperature which should never exceed 45 °C. The optical rotation of **2b** obtained by this method was $[\alpha]^{20}_D = +143.2°$. The optical purity was thus practically 100%. Subsequent transformations to produce **9b** were carried out in an analogous manner.

Structural Confirmation

The intermediates and final products obtained were unambiguously characterized by infrared, ^{1}H-NMR, and mass spectroscopic investigation.

The spectroscopic results obtained with the diastereomers **8a,b** and the final products **9a,b** for the determinations of the diastereomeric or enantiomeric purities are of particular significance. Since it is known that diastereomers generate a double set of signals in the ^{1}H-NMR spectrum, a determination of the relative proportions in the diastereomeric mixture is possible by an exact signal differentiation and comparison of signal intensities. This differentiation can be achieved on the basis of the signals of the methoxy group in the side chain at $\delta = 3.2$ ppm in the 400-MHz spectrum. If **8** is optically pure, only one signal can be detected, whereas a diastereomeric mixture causes two signals. In order to assure the configurative homogeneity of **9a,b**, the addition of chiral lanthanide shift reagents should give rise to the formation of diastereomeric complexes, and this should induce a signal splitting for those groups located in the vicinity of the asymmetric center.[23] Good results were obtained by the 50% addition of the tris[3-(heptafluoropropylhydroxymethylene)-(+)-camphorato]praesodymium(III) complex which clearly splits the signal of the ester methyl group of **9** while simultaneously producing a highfield shift to $\delta = 2.8$–2.9 ppm. The optical antipodes **9a** and **9b** each exhibit only one signal in this region; the enantiomeric excess can thus be given as ee >98%.

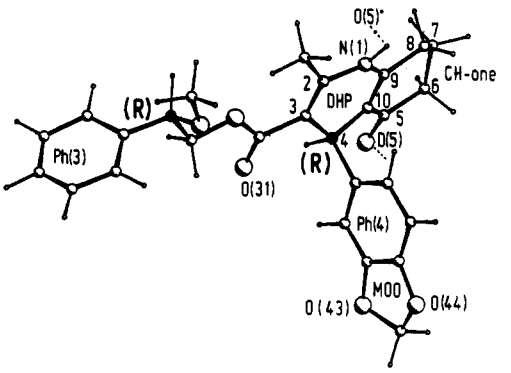


Figure 1. PLUTO-78 drawing of **8a**, the (*R,R*)-diastereomer of **8** as found by structure analysis. Atom C(7) is located in two positions; the main component (67%) is shown.

Table I. Investigations on KCl-Stimulated Aortic Strips of Guinea Pigs: EC_{50} Value and Intrinsic Activity

compound	EC_{50} (mol/L)	max effect (%)[a]	IA[b]	n[c]
nifedipine	8.9×10^{-9}	70 ± 6.6	1	4
9a	1.0×10^{-5}	38 ± 1.6	0.54	3
9b	2.6×10^{-7}	84 ± 3.8	1.2	5
racemate **9**	2.0×10^{-6}	73 ± 3.1	1	5
8a	4.0×10^{-5}	42 ± 2.1	0.6	4
8b	7.1×10^{-6}	70 ± 5.3	1	5

[a] Contraction inhibition ± SEM. [b] Nifedipine = 1. [c] Number of experiments.

Determination of the Absolute Configuration of 8a. Figure 1 shows the **8a** molecule found by X-ray structure analysis. It unequivocally contains the (*R,R*)-configuration. The hexahydroquinolinone core of the molecule exhibits fully normal geometry apart from the fact that one of the methylene groups is located in two positions. Obviously, the cyclohexenone ring is somewhat nonrigid. The conformations of the "three" six-membered rings are dihydropyridine flat boat (nearly C_s symmetric with a mirror plane through the atoms N(1) and C(4)), main component of cyclohexenone half chair (nearly C_s symmetric with a mirror plane through atoms C(10) and C(7)), and secondary component of cyclohexenone half-boat (nearly C_s symmetric with a mirror plane through atoms C(10) and C(7a)). The five-membered dioxacyclopentene ring has an envelope conformation (nearly C_s symmetric with a mirror plane through the bond C(43)–C(44) and through the top atom C(47)). All other bond lengths and angles of **8a** span the normal limits and merit no special comment. Noteworthy is a hydrogen bond from N–H to the carbonyl group of the cyclohexenone of a neighboring molecule (H···O 2.04 Å). This hydrogen bond is slightly bent (144°) and has a local anti conformation (−177°). Overall, the hydrogen bond results in helical chains of the **8a** molecules along the *y* axis of the unit cell (space group $P2_12_12_1$). The existence of the hydrogen bond is confirmed by infrared spectroscopy.[11]

Pharmacology

Similar to the racemate,[11,14] the effects of the optically pure hexahydroquinolinones **9a,b** and the diastereomeric

(21) Neilson, D. G.; Peters, D. A. V. A New Resolution of α-Methoxy-α-phenylacetic Acid. *J. Chem. Soc.* **1962**, 1519.
(22) Kirmse, W.; Plath, P.; Schaffroth, H. Stereochemistry of Aliphatic Carbonium Ions. 8. Rearrangements of the 2-Methoxy-2-phenylethyl System. *Chem. Ber.* **1975**, *108*, 79–87.
(23) Friebolin, H. One- and Two-Dimensional NMR Spectroscopy; Verlag Chemie: Weinheim, 1988; p 275.

Table II. Investigations on $BaCl_2$-Stimulated Ileum of Guinea Pigs: EC_{50} Value and Intrinsic Activity

compound	EC_{50} (mol/L)	max effect (%)[a]	IA[b]	n[c]
nifedipine	2.0×10^{-8}	95 ± 2.8	1	9
9a	2.5×10^{-5}	42 ± 5.9	0.44	3
9b	2.2×10^{-5}	89 ± 5.1	0.94	4
racemate **9**	5.0×10^{-5}	76 ± 5.9	0.8	4

[a] Contraction inhibition $\bar{x}$ ± SEM. [b] Nifedipine = 1. [c] Number of experiments.

intermediates **8a,b** were also investigated on isolated guinea pig organs. For determination of the effects on smooth musculature, KCl-stimulated aortic strips and $BaCl_2$-stimulated ileum were used while electrically stimulated left atria and papillary muscles were employed for cardiac activities. The data obtained from these tests are shown in Tables I and II.

Since calcium channel blockers are not antagonists in the usual pharmacological sense but rather reduce the frequency and duration of channel openings by direct activation at DHP receptors, the term "intrinsic activity" has been introduced to describe the maximum degree of activity.

Both antipodes **9a** and **9b** exhibited calcium antagonistic effects on smooth musculature although, particularly for aortic preparations, the EC_{50} and maximum degree of activity values differ markedly. The maximum contraction inhibition of the (*S*)-(−)-enantiomer **9b** was 84%, representing a relative degree of activity of 1.2 compared with the standard nifedipine (maximum degree of activity: 70%). A comparison of the EC_{50} values showed an approximately 10-fold higher activity of the (*S*)-(−)-enantiomer compared with the racemate and a 100-fold higher activity compared with the (*R*)-(+)-enantiomer.

In the same manner, the optically pure diastereomers **8a,b** exerted differing activities on smooth vascular musculature (Table I), with the (*S,S*)-(−)-diastereomer **8b** being the more potent compound. Owing to the enlargement of the ester side chain the effects were less pronounced than those of the enantiomers **9a,b**.

The racemate of **9** exhibited a positive inotropic effect on the electrically stimulated left atrium which was not suppressed by treatment with propranolol hydrochloride or procaine hydrochloride.[11] However, these activities were not reproducible on ventricular papillary muscle.[14] Although the (*S*)-(−)-enantiomer **9b** was the more potent component on smooth musculature, it exhibited only a relatively weak positive inotropic activity on electrically stimulated left atrium (maximum increase $\bar{x}$ = 69% ± 4.6, intrinsic activity (ia) = 0.15, compared with the standard CGP 28392, $EC_{50} = 5.6 \times 10^{-5}$, $n = 5$), and this activity disappeared on pretreatment with propranolol hydrochloride (5×10^{-6} mol/L bath concentration). In contrast, the (*R*)-(+)-enantiomer **9a** produced a more pronounced increase in contractility which was only slightly suppressed by pretreatment with propranolol hydrochloride (maximum increase $\bar{x}$ = 120% ± 12.0, ia = 0.3, compared with the standard CGP 28392, $EC_{50} = 9.4 \times 10^{-7}$, $n = 6$).

In comparison with the standard agonist CGP 28392, the intrinsic activity value of **9a** was 0.3 while the EC_{50} values are approximately equal. Like the racemate, the enantiomers did not exert any observable effect on electrically stimulated papillary muscle. The diastereomers **8a,b** showed only weak positive inotropic activities on the atrium.

Discussion

A route for the preparation of enantiomerically pure hexahydroquinolinones comprising a diastereoselective synthesis in which a chiral acetoacetate provides for optical induction has been developed. Determination of the absolute configuration was performed by using the method of single crystal X-ray analysis.

The results of pharmacological investigations clearly confirmed the stereoselectivity of DHP receptors since the EC_{50} values of the antipodes on smooth musculature (KCl-stimulated aortic strips) differed by a factor of 100 while the degrees of activity were also clearly different.

Although the racemate exhibited positive inotropic effects on left atria, these activities were not reproducible on ventricular papillary muscle.[11,14] A possible explanation for this observation could be a lengthening of the duration of the action potential at the atrium. Such a phenomenon is also known for papaverine,[24] which exerts a positive inotropic effect on isolated left atrium but not on papillary muscle. However, since a rightward shift of the nifedipine dosage–activity curve was observed in spite of pretreatment with the test substance, the increase in contractility could possibly be caused by a dualistic effect. In any case, the effects on the atria are apparently stereoselective since the (*R*)-enantiomer, which has a markedly weaker effect on smooth musculature, is the active form in the present case. This observation also supports the assumption that the cardiac effect is, at least partly, mediated by DHP receptors. Further investigations on enantiomerically pure substances of the types **10**, **11**, and **12** should provide more information for the clarification of these effects.

Experimental Section

1. **Pharmacology. (a) Investigations on Isolated, Electrically Stimulated, Left Atria of Guinea Pigs.** Healthy guinea pigs of either six with body weights of 250–450 g were killed by a blow to the neck and exsanguinated. After opening of the thorax, the hearts were removed and immediately transferred to a Tyrode solution at 37 °C under perfusion with carbogen (95% O_2, 5% CO_2). The left atria were prepared and fixed on a DMS detector type TF 6 V5 (Fleck, Mainz, FRG) in an organ bath of 50-mL capacity between two platinum electrodes (Fleck, Mainz/FRG). The liquid in the bath consisted of a carbogen-perfused Tyrode solution at 37 °C having the following composition: NaCl 10.0, KCl 0.25, $CaCl_2 \cdot 2H_2O$ 0.33, $MgCl_2 \cdot 6H_2O$ 0.27, $NaHCO_3$ 1.0, $NaH_2PO_4 \cdot 2H_2O$ 0.65, glucose 1.0 g in 1000 mL of H_2O (pH 7.7). The electrical stimulation was performed at a frequency of 1 Hz with square wave impulses of 2 ms and a potential 20% over the threshold. The isometrically measured impulses under a preloading of 0.5 g were recorded on a direct recorder after electrical amplification (Hellige TF 19). After an equilibration period of 30 min, the concenrtation–activity curves of the individual substances to be tested were recorded using the cumulative technique[25] (seven values in the dose range 1×10^{-7} to 1×10^{-4} mol/L). In order to investigate the DHP receptor selectivity, concentration–activity curves for nifedipine in the presence and absence of the agonists under testing were also determined. A fresh organ was used for each experiment; for the number of experiments, see Tables I and II.

(b) Investigations on Isolated, Electrically Stimulated, Papillary Muscles of Guinea Pigs. Healthy guinea pigs of either sex with body weights of 250–450 g were killed by a blow to the neck and exsanguinated. After opening of the thorax, the hearts were removed and immediately transferred to a Tyrode solution (for composition, see above) at 37 °C under perfusion with carbogen (95% O_2, 5% CO_2). After opening of the right ventricle, a papillary muscle was prepared and fixed on a DMS

(24) Nawrath, H.; Meinertz, T. Electrical and Mechanical Activity of Mammalian Heart Muscle Fibres Treated with Papaverine. *Naunyn Schmiedeberg's Arch. Pharmacol.* **1977**, *299*, 253–258.
(25) Van Rossum, J., M. Cumulative Dose-Response Curves. II. Technique for Making Dose-Response Curves in Isolated Organs and the evaluation of Drug Parameters. *Arch. Int. Pharmacodyn. Ther.* **1963**, *143*, 299–330.

detector type TF 6 V5 (Fleck, Mainz, FRG) in an organ bath of 50-mL capacity between two platinum electrodes (Fleck, Mainz, FRG). The liquid in the bath consisted of a carbogen-perfused Tyrode solution at 37 °C. The electrical stimulation was performed at a frequency of 1 Hz with square wave impulses of 3 ms and a potential 20% over the threshold. The isometrically measured impulses under a preloading of 0.2 g were recorded on a direct recorder after electrical amplification (Hellige TF 19). After an equilibration period of 30 min, the concentration–activity curves of the individual substances to be tested were recorded using the cumulative technique[25] (seven values in the dose range 1×10^{-7} to 1×10^{-4} mol/L). A fresh organ was used for each experiment.

(c) Investigations on Isolated Ileum of Guinea Pigs. Healthy guinea pigs of either sex with body weights of 250–450 g (see under 1a above) were killed by a blow to the neck and exsanguinated. After opening of the abdominal cavity, parts of the small intestine were removed, freed from mesenteric tissue, and stored for 3 h in Tyrode solution (for composition, see above) at 37 °C under perfusion with carbogen. Subsequently, 3-cm lengths of intestine were fastened to a DMS detector type TF3 V3 (Fleck, Mainz, FRG) (preloading 0.56 g) in an organ bath of 50-mL capacity. Measurement of the contraction effects was achieved after amplification using a Hellige preamplifier TF 19 with the aid of a Rika-Denki electronic recorder (three-pen recorder). The liquid in the bath again consisted of carbogen-perfused Tyrode solution at 37 °C. In order to check for antagonistic effects, contractions were induced with barium chloride (4×10^{-3} mol/L bath concentration). After thorough washing out, this process was repeated until the amplitude of the contraction became constant. Investigations of the substances to be tested were performed using the single-dose technique in which the $BaCl_2$ contractions were induced after addition of the test substance and 10-min exposure time. Between administrations of the individual substances, the preparation was washed until the initial situation had been reestablished and the $BaCl_2$ contractions were then induced.

(d) Investigations on Isolated Aortic Strips of Guinea Pigs. The abdominal cavities of healthy guinea pigs were opened, and the aortas were removed and freed of adhering tissue. The aortas were cut in spirals and 3–4-cm long strips were fastened to a DMS detector type TF6V5 (Fleck, Mainz, FRG) in an organ bath of 50-mL capacity. The liquid in the bath consisted of a carbogen-perfused Tyrode solution at 37 °C. The isometrically measured contraction effects under a preloading of 2 g were recorded on a Rika-Denki electronic recorder after amplification using a Hellige preamplifier TF 19. In order to check for antagonistic effects, contractions were induced with potassium chloride (67 mmol/L bath concentration). After thorough washing out, this process was repeated until the amplitude of the contraction became constant. Investigations of the substances to be tested were performed using the single dose technique in which the KCl contractions were induced after addition of the test substance and 10-min exposure time. Between administrations of the individual substances, the preparation was washed until the initial situation had been reestablished and the KCl contractions were then induced. (EC_{50} values and intrinsic activity as under 1c above.)

2. Chemistry. Melting points were taken with a Büchi SMP 20 melting point apparatus and are uncorrected. Infrared spectra were taken with Beckmann IR-33 and IR-4220 spectrophotometers. ^{1}H-NMR data were obtained with Varian EM 360 and Bruker AM 400 spectrometers using TMS as internal standard. Mass spectra were taken with a Varian MAT CH 7A (Bremen, FRG). TLC, CC, and PCC were performed on Merck silica gel of various activity grades. The structures of all compounds were substantiated by their IR, ^{1}H-NMR, and mass spectral data. All compounds were analyzed for C, H, N, and the results were within 0.4% of the calculated values.

(*R*)-(−)-Methoxyphenylacetic Acid (2a). (a) To a warm (40 °C) solution of 85.0 g (2.14 mol) of NaOH in 400 mL of water was added 25.0 g (0.16 mol) of (*R*)-(−)-mandelic acid (1a). While being stirred, 111.0 g (0.88 mol) of dimethyl sulfate was added dropwise at 45 °C (exact control of temperature) within 3 h. After cooling, the formed precipitate was filtered off and dissolved in hot water. The pH value was adjusted to 0 by addition of concentrated HCl, and the resulting oil was repeatedly extracted with diethyl ether. The combined diethyl ether phase was dried and concentrated, and the oily residue was recrystallized from petroleum ether (80–120 °C) to produce colorless needles with mp 70 °C. Yield: 8.2 g (31%). $[\alpha]^{20}_D$: −143.9° (*c* = 1, ethanol) [Lit.[22]: $[\alpha]^{21}_D$ = −144° (ethanol). Lit.[21]: $[\alpha]^{22}_D$ = −140.0° (ethanol)].

(b) Following the method described above, racemic **2** was obtained from 80.0 g (0.526 mol) of (*RS*)-mandelic acid, 278 g (6.96 mol) of NaOH, and 364.5 g (2.89 mol) of dimethyl sulfate at 50 °C. Yield: 40.8 g (47%). Fifty grams (0.248 mol) of ephedrine hydrochloride and 40 g (0.248 mol) of NaOH were dissolved in 300 mL of water, and the liberated base was extracted with diethyl ether. An amount of 34.2 g (0.207 mol) of ephedrine and 35.0 g (0.21 mol) of (*RS*)-**2** were dissolved in 170 mL of hot ethanol. After the solution was allowed to cool to room temperature, the resulting crystals were filtered off and repeatedly recrystallized from ethanol. The colorless needles were dissolved in 150 mL of water, and the solution was treated with dilute HCl and extracted with diethyl ether. The diethyl ether phases were combined, dried, and concentrated. The resulting oil was purified as described under 2a above to produce colorless needles. Mp: 70 °C. Yield: 9.5 g. $[\alpha]^{20}_D$: −142.2°. Anal. ($C_9H_{10}O_3$) C, H, N.

(*S*)-(+)-Methoxyphenylacetic Acid (2b). **2b** was prepared as described for **2a** from 31.4 g (0.207 mol) of *S*-(+)-mandelic acid (**1b**), 110 g (2.76 mol) of NaOH, and 143.3 g (1.14 mol) of dimethyl sulfate. Yield: 12.8 g (37%). $[\alpha]^{20}_D$: +143.2° (*c* = 1, ethanol). Anal. ($C_9H_{10}O_3$) C, H, N.

(*R*)-(−)-2-Methoxy-2-phenylethanol (3a). In 100 mL of anhydrous diethyl ether 6.3 g (65 mmol) of lithium aluminum hydride was suspended, and then a solution of 9.5 g (57 mmol) of **2a** in 50 mL of diethyl ether was slowly added dropwise at room temperature. After 2 h, the mixture was carefully hydrolyzed and repeatedly boiled with diethyl ether, and the combined diethyl ether phases were dried with calcium chloride. Concentration of the diethyl ether phases gave a colorless oil. Yield: 7.7 g (89%). n^{20}_D: 1.5149. $[\alpha]^{20}_D$: −129.5° (neat) [lit.[22]: $[\alpha]^{21}_D$ −126.6° −126.0 (neat). Lit.[21]: $[\alpha]^{22}_D$ = −125.9° (neat)]. Anal. ($C_9H_{12}O_2$) C, H, N.

(*S*)-(+)-2-Methoxy-2-phenylethanol (3b). **3b** was prepared analogously to **3a** from 12.8 g (77 mmol) of **2b** and 7.5 g (77 mmol) of lithium aluminum hydride in 100 mL of diethyl ether. Yield: 9.1 g (78%). $[\alpha]^{20}_D$: +126.9°. Anal. ($C_9H_{12}O_2$) C, H, N.

(*R*)-(−)-2-Methoxy-2-phenylethyl Acetoacetate (5a). In 150 mL of toluene 7.4 g (48.6 mmol) of **3a** and 4.63 g (55 mmol) of diketene (**4**) were dissolved. An amount of 0.8 g of sodium acetate was added, and the mixture was heated under reflux at 90 °C for 4 h. After concentration of the reaction mixture under vacuum, the resulting brown oil was purified by flash chromatography on silica gel (mobile phase: dichloromethane) to produce a colorless oily liquid. Yield: 5.3 g (46%). $[\alpha]^{20}_D$: −64.8° (*c* = 1, ethanol). n^{20}_D: 1.4908. Anal. ($C_{13}H_{16}O_4$) C, H, N.

(*S*)-(+)-2-Methoxy-2-phenylethyl Acetoacetate (5b). **5b** was prepared analogously to **5a** above from 7.0 g (46 mmol) of **3b** and 6.55 g (46 mmol) of diketene–acetone adduct in 150 mL of toluene with heating at 100 °C for 5 h and flash chromatographic purification. Yield: 5.3 g (49%). $[\alpha]^{20}_D$: +63.3° (*c* = 1.0, ethanol). Anal. ($C_{13}H_{16}O_4$) C, H, N.

(*R*,*R*)-(+)-2-Methoxy-2-phenylethyl 2-Methyl-4-[3,4-(methylenedioxy)phenyl]-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (8a). In 100 mL of ethanol 3.0 g (12.7 mmol) of **5a**, 1.9 g (12.7 mmol) of 3,4-(methylenedioxy)benzaldehyde (**7**), and 1.42 g (12.7 mmol) of 1,3-cyclohexanedione (**6**) were dissolved. Five milliliters of 25% ammonia solution and 5 mL of concentrated acetic acid were added, and the resulting mixture was heated to boiling for 2.5 h. The crude product which precipitated upon cooling was filtered off and recrystallized from methanol to produce colorless needles of mp 234 °C. Yield: 1.1 g (19%). $[\alpha]^{20}_D$: +59.5° (*c* = 1.0, DMSO). ^{1}H-NMR ($CDCl_3$): δ (ppm) 1.9–2.0 (m, CH_2-7), 2.29 (s, 3 H, CH_3 at C-2), 2.3–2.5 (m, 4 H, CH_2-6, CH_2-8), 3.23 (s, 3 H, OCH_3), 4.13 (d, 2 H, $COOCH_2$, *J* = 7 Hz), 4.3 (t, 1 H, $COOCH_2CH$, *J* = 7 Hz), 5.02 (s, 1 H, *H* at C-4), 5.84 (s, split, 2 H, CH_2 of methylenedioxy group), 6.04 (s, 1 H, N*H*, exchangeable with D_2O), 6.61 (d, 1 H, *H*-5 of substituted phenyl ring, ^3J = 8 Hz), 6.72 (d, split, 1 H, *H*-6 of substituted phenyl ring, ^3J = 8 Hz), 6.78 (s, split, 1 H, *H*-2 of substituted phenyl ring), 7.25–7.35 (m, 5 H, *H* of phenyl ring of ester side

Table III. Crystallographic Data for 8a and Structure Determination Details

formula, M	$C_{27}H_{27}NO_6$, 461.52
crystal system, space group	orthorhombic, $P2_12_12_1$ (No. 19)
unit cell dimensions	
a	8.222 (2) Å
b	12.568 (3) Å
c	22.736 (5) Å
packing: V, Z, $F(000)$	2349 (1) Å^3, 4, 976
D_{calcd}, D_{exptl}	1.305, 1.309 g cm^{-3}
reflctns: measd, indep (int R)	5806, 2903 Fp (0.0616)
reflctns used, limit	2276 (1138 Fp) with $I > 1\sigma(I)$
var, ratio Fp/var, last shifts	318, 3.6, <0.03 σ
final R, R_w	0.0908, 0.0501
weighting scheme w^{-1}	$\sigma^2(F) + 0.000179F^2$

chain). Anal. ($C_{27}H_{27}NO_6$) C, H, N.

(*S*,*S*)-(−)-2-Methoxy-2-phenylethyl 2-Methyl-4-[3,4-(methylenedioxy)phenyl]-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (8b). 8b was prepared analogously to 8a above from 1.0 g (4.2 mmol) of 5b, 0.64 g (4.2 mmol) of 7, and 0.48 g (4.2 mmol) of 6 in 80 mL of ethanol with addition of 4 mL of 25% ammonia solution and 2 mL of concentrated acetic acid. Colorless crystals from methanol with mp 215 °C. Yield: 0.3 g (15%). $[\alpha]^{20}_D$: −60.5° (c = 1, DMSO). Anal. ($C_{27}H_{27}NO_6$) C, H, N.

(*R*)-(+)-Methyl 2-Methyl-4-[3,4-(methylenedioxy)phenyl]-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (9a). 9a was obtained by dissolving 0.8 g (1.7 mmol) of 8a in 80 mL of methanol, adding 1.0 g (44 mmol) of metallic sodium, and heating the resultant mixture to boiling for 5.5 h. The mother liquor was then concentrated to half its original volume, treated with dilute HCl, and diluted with 70 mL of water. After being allowed to cool, the mixture was extracted repeatedly with dichloromethane, and the combined dichloromethane phases were combined and concentrated to produce a yellowish oil. Crystallization from methanol produced colorless needles with mp 250 °C. Yield: 0.4 g (67%). $[\alpha]^{20}_D$: +148.6° (c = 1.0, DMSO). Anal. ($C_{19}H_{19}NO_5$) C, H, N.

(*S*)-(−)-Methyl 2-Methyl-4-[3,4-(methylenedioxy)phenyl]-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (9b). 9b was prepared analogously to 9a above from 0.28 g (0.6 mmol) of 8b and 0.35 g (15 mmol) of sodium in 50 mL of methanol with a reaction time of 6 h. Workup as described for 9a furnished colorless needles of mp 249 °C. Yield: 0.1 g (49%). $[\alpha]^{20}_D$: −151.7° (c = 1, DMSO). Anal. ($C_{19}H_{19}NO_5$) C, H, N.

3. X-ray Structure Analysis of 8a. Crystal data as well as details of intensity data collection and refinement are given in Table III. The density was obtained from neutral buoyancy (Thoulet solution). The crystal was fixed by glue on a glass fiber and sealed in a glass capillary tube. The quality and symmetry of the crystal was examined by Weissenberg exposures. Integrated intensities were measured by means of $\omega/2\theta$ scans on a CAD4 diffractometer (Enraf-Nonius).

The structure was solved by direct methods (SHELX-86). The refinement (anisotropic temperature factors for all non-hydrogen atoms except the p-phenyl atom C(344)) was by full matrix. Hydrogen positions were considered as riding on carbon atoms except the hydrogen atoms at nitrogen and at the two chiral centers: the latter three hydrogen atoms were refined with isotropic temperature motion. Afterwards, one of the methylene groups of the cyclohexenone rings exhibited a strong thermal motion and a grossly distorted geometry. By means of several difference Fourier syntheses it was possible to split this methylene group in two positions below and above the ring). The ratio of 67/33 for these two positions was chosen by attaining the same isotropic motion in the refinement.

In spite of this consideration and in spite of a careful and long measurement of the Friedel pairs, it was not possible to determine the absolute configuration of 8a without any additional information. This additional information arose from the mandelic acid (R)-center of 8a, and by choosing this center, the second center came out with (R)-configuration as well.

The final refinement came out with a good convergence and an even distribution of the variances. Besides several local written routines, local versions of SHELX-76 and SHELX-86 were used for the calculations and a local version of PLUTO-78 was used for the figure (HB-DPS-8/70 equipment at Zentrum für Datenverarbeitung, Universität Mainz).

Registry No. (*RS*)-1, 611-72-3; 1a, 611-71-2; 1b, 17199-29-0; (*RS*)-2, 7021-09-2; 2a, 3966-32-3; 2b, 26164-26-1; 3a, 17628-72-7; 3b, 66051-01-2; 4, 674-82-8; 5a, 104451-36-7; 5b, 77940-85-3; 6, 504-02-9; 7, 120-57-0; 8a, 139758-84-2; 8b, 139758-85-3; 9a, 139758-86-4; 9b, 139758-87-5.

Supplementary Material Available: Table I with noteworthy bond lengths and angles, Table II with fractional atomic coordinates and equivalent isottropic thermal parameters, and tables with anisotropic thermal parameters, H atom coordinates, and a complete listing of bond distances and angles (5 pages); observed and calculated structure factor amplitudes (13 pages). Ordering information is given on any current masthead page.

Selective Reversible and Irreversible Ligands for the κ Opioid Receptor

Chen-Yu Cheng,* Shou-Chien Wu, Ling-Wei Hsin, and S. William Tam†

School of Pharmacy, College of Medicine, National Taiwan University, 1, Section 1, Jen-Ai Road, Taipei, Taiwan 10018, and Central Nervous System Diseases Research, The Du Pont Merck Pharmaceutical Company, Wilmington, Delaware 19880-0400.
Received January 22, 1992

(±)-(5β,7α,8β)-3,4-Dichloro-*N*-methyl-*N*-[3-methylene-2-oxo-8-(1-pyrrolidinyl)-1-oxaspiro[4,5]dec-7-yl]benzeneacetamide (14) and its (5α,7α,8β) diastereomer 15 have been synthesized from 1,4-cyclohexanedione monoethylene ketal (1) in 10 steps. Compound 14, which we have designated SMBU-1, was found to bind with moderate affinity (K_i = 109 nM) and good selectivity (μ/κ = 29) to the κ opioid receptor, while 15 was only 1/10 as potent as a κ ligand. Preincubation of brain membranes with 14 resulted in wash-resistant inhibition of κ-receptor binding (69 ± 6% of control at 10^{-6} M). The ketone precursor *trans*-*N*-methyl-*N*-[5-oxo-2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide (12) showed a higher κ-affinity (K_i = 78 nM) and a much higher κ-selectivity (μ/κ = 166) than 14. Compound 10, the ethylene ketal precursor of 12, exhibited a similar receptor binding profile to 14, with increased κ-selectivity (μ/κ = 55), while ketal 11, being a regioisomer of 10 and an oxygen isostere of the κ-selective analgesic spiradoline (U-62,066), demonstrated the highest κ-affinity (K_i = 1.5 nM) and κ-selectivity (μ/κ = 468) observed in this series.

Since the initial proposals of multiple opioid receptors,[1,2] the existence of at least three different types of opioid receptor, namely, μ, κ, and δ, has been well established.[3-5] In recent years, the discovery of irreversible opioid ligands,

* To whom correspondence should be addressed at the School of Pharmacy, College of Medicine, National Taiwan University, 1, Section 1, Jen-Ai Rd., Taipei, Taiwan 10018.
† The Du Pont Merck Pharmaceutical Co.

(1) Portoghese, P. S. A New Concept on the Mode of Interaction of Narcotic Analgesics with Receptors. *J. Med. Chem.* 1965, *8*, 609.

0022-2623/92/1835-2243$03.00/0